Current Status of Src Inhibitors in Solid Tumor Malignancies

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ABSTRACT

Summary. Src is believed to play an important role in cancer, and several agents targeting Src are in clinical development.

Design. We reviewed Src structure and function and preclinical data supporting its role in the development of cancer via a PubMed search. We conducted an extensive review of Src inhibitors by searching abstracts from major oncology meeting databases in the last 3 years and by comprehensively reviewing ongoing clinical trials on ClinicalTrials.gov.

Results. In this manuscript, we briefly review Src structure and function, mechanisms involving Src that lead to the development of cancer, and Src inhibitors

and key preclinical data establishing a rationale for clinical application. We then focus on clinical data supporting their use in solid tumor malignancies, a newer arena than their more well-established hematologic applications. Particularly highlighted are clinical trials investigating new biomarkers as well as ongoing studies assessing Src inhibitor activity in biomarker-selected patient populations. We also review newer investigational Src-targeting agents.

Conclusions. Src inhibitors have shown little activity in monotherapy trials in unselected solid tumor patient populations. Combination studies and biomarker-driven clinical trials are under way. *The Oncologist* 2011;16:566–578

INTRODUCTION

Src was the first proto-oncogene identified from the human genome and encodes a non-receptor tyrosine kinase (RTK). The origins of the discovery of Src began in the early 1900s when Francis Peyton Rous proposed that viruses could cause cancer [1]. He injected centrifuged chicken sarcoma material into chicks that later developed sarcomas themselves. This cancer-causing substance was

later found to contain a retrovirus, later named the Rous sarcoma virus [2]. The gene implicated, viral-Src (*v-Src*), was identified in 1970 [3]. Bishop and Varmus won the Nobel Prize in 1989 for their work showing that the virus actually acquired the cancer-causing gene from a normal cellular gene, cellular-Src (or *c-Src*, hereafter referred to as *Src*) [4–6]. In normal tissues, Src's regulatory functions are multiple and include participation in fibroblast cell division and

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cell–cell adhesion regulation via modulation of integrins. Consequently, its overexpression is believed to play an important role in cancer progression, and several agents targeting Src are in clinical development. In this manuscript we review Src-targeting agents, focusing on new data in solid tumor malignancies.

The Src protein is one of the Src family kinases (SFKs), reviewed in detail by both Yeatman [7] and Thomas and Brugge [8]. There have been 12 SFKs identified: c-Src, Fyn, Yes, Yrk, Lyn, Hck, Fgr, Blk, Lck, Brk, Srm, and Frk (with Frk/Rak and Iyk/Bsk subfamilies), 11 of which are found in humans [8–12]. Src, Fyn, and Yes are expressed ubiquitously, with Src being expressed at five to 200 times higher levels in platelets, neurons, and osteoclasts [13]. The Src protein consists of a 12-carbon myristoyl group attached to the N-terminal domain, followed by a unique domain, SH3 and SH2 domains, an SH2-kinase linker domain, a protein kinase catalytic domain (or SH1 domain), and a negative regulatory C-terminal tail domain [7]. The SH3 domain is noncatalytic and thought to mediate protein–protein interactions responsible for localization and recruitment of substrates [8]. SH2 interacts with proteins such as focal adhesion kinase (FAK) and CRK associated substrate (CAS). The unique domain shows the greatest amount of sequence divergence among family members whereas the SH1 catalytic domain is well conserved.

Src is a non-RTK, and its activity mainly occurs at the inner leaflet of the cell because membrane localization is required for receptor-mediated signaling to occur. Src is thought to facilitate motility and invasion of tumor cells by promoting endocytosis of cell–cell adhesions [14], mediating assembly and disassembly of focal adhesions [15], and regulating expression of matrix metalloproteinases (MMPs) that contribute to breakdown of the extracellular matrix [16, 17]. Src is also thought to activate signal transducer and activator of transcription (STAT)-3, an important protein that mediates angiogenesis through vascular endothelial growth factor (VEGF) activation [18].

Src interacts with several important RTKs, including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor, hepatocyte growth factor receptor, colony stimulating factor-1 receptor (CSF-1R), insulin-like growth factor-1 receptor (IGF-1R), human epidermal growth factor receptor 2/Neu, and stem cell factor receptor (c-Kit) [19–26]. The relationship of Src and the transmembrane RTKs is a complex, bidirectional process [27]. When the RTKs are activated, several intracellular proteins are necessary for signal propagation to the nucleus, and Src is one of these important protein mediators [8]. Src also acts to modulate the function of the RTKs [8]. EGFR and Src, for instance,

appear to have a synergistic effect whereby higher levels of the combination of the two proteins demonstrate greater tumorigenesis than with either alone [28–31].

INVOLVEMENT OF SFKS IN CANCERS

SFKs have been shown to be involved in numerous human cancers (reviewed in detail by Summy and Gallick [32]). Colon cancer is the most extensively studied cancer related to Src, but others identified include breast cancer, lung cancer, pancreatic cancer, gastric cancer, ovarian cancer, bladder cancer, head and neck cancer, esophageal cancer, brain cancer, melanoma, Kaposi's sarcoma, and lymphoproliferative disorders (myelomas, leukemias, lymphomas) [32, 33].

In colon cancer, an early study by Bolen et al. [34] showed elevated Src activity in colonic carcinoma cell lines and tumors. Higher levels of kinase activity were noted in primary colonic tumors than in precancerous polyps, and a similar result was found for hepatic metastases versus primary tumors [35]. However, despite these findings, some evidence shows that Src kinase activity is actually higher in the earlier stages of cancer progression. The discrepancy of these data could be explained by the observation that Src contributes to the metastatic phenotype but is not required at all stages of cancer progression [32]. Despite this discrepancy, Src appears to be an independent prognostic indicator at all stages of colon carcinoma [36]. For upper gastrointestinal tumors, Src kinase activity was higher in eight of 10 gastric carcinomas than in normal mucosa [37], and three- to six-fold higher in Barrett's esophagus and esophageal adenocarcinomas than in controls [38, 39]. Several other cancers also show involvement by either Src or other SFK members. Src expression was found in 20 of 33 tumor samples of small cell lung cancer and non-small cell lung cancer (NSCLC), in 60%–80% of adenocarcinomas and bronchioalveolar carcinomas and 50% of squamous cell carcinomas. Poorly differentiated lung cancers had higher Src expression levels than moderately to well-differentiated ones [40].

Several studies have demonstrated involvement of SFKs in the development of breast cancer as well. Elevated levels of Src have been noted in breast carcinoma cell lines [41, 42]. Src also plays a role in ErbB-2–mediated breast cancer, for which two mechanisms have been proposed. One mechanism involves ErbB-2 upregulation of Src translation by activating the Akt/mammalian target of rapamycin/eukaryotic translation initiation factor 4E-binding protein 1 pathway. The second describes stabilization of Src through inhibition of calpain protease–mediated Src degradation, leading to upregulated Src protein levels. Interestingly, Src inhibition was associated with dramatically

lower ErbB-2-mediated breast cancer cell invasion in vitro and in animal models [43].

Src protein levels were also found to be elevated in both pancreatic tumors and cell lines [44]. Src inhibitors inhibit the growth of human pancreas tumor explants in preclinical models [45, 46]. Activated Src also increases levels of IGF-1R with a subsequent increase in IGF-dependent cell proliferation [47].

SRC INHIBITORS

With the wealth of literature supporting the role of Src in tumor progression, invasion, and metastasis, efforts have been under way in developing inhibitors of this non-RTK. Different classes of Src inhibitors have been developed, with several of these drugs showing promise in completed and ongoing clinical trials. Although selective Src inhibitors have been developed, dual inhibitors of Src and Bcr-Abl have been the compounds investigated for efficacy in patients. One such inhibitor, dasatinib, has U.S. Food and Drug Administration (FDA) approval for second-line therapy in chronic myelogenous leukemia patients. Here, we focus on the application of these inhibitors in the treatment of patients with solid tumors.

Dasatinib

Dasatinib (BMS-354825; Bristol-Myers Squibb, New York) is an orally bioavailable, ATP-site competitive 2-(aminopyrimidinyl)thiazole-5-carboxamide that is the only FDA-approved Src-Abl inhibitor for use in patients with chronic myelogenous leukemia (CML) or Philadelphia chromosome positive (Ph⁺) acute lymphoblastic leukemia (ALL) failing first-line therapy with imatinib. Recently, in a study of 512 patients with newly diagnosed chronic-phase CML, Kantarjian et al. [48] demonstrated that, compared with imatinib as first-line treatment, dasatinib achieved higher rates of complete cytogenetic response, response in a shorter time, and lower rates of progression to accelerated or blastic phase. It is the most well-studied Src-Abl inhibitor, displaying a 325-fold greater potency for Bcr-Abl inhibition than imatinib. It is fairly unselective, inhibiting an array of additional downstream tyrosine kinases including c-Kit, c-FMS, ephrin type-A receptor 2 (EphA2), and PDGFR. The contribution of other kinase inhibitory activity to clinical efficacy remains a significant unknown (Table 1).

Preclinical data demonstrated the activity of dasatinib against several solid tumors. Src pathway inhibition has been shown in prostate cancer, head and neck cancer, NSCLC, colon cancer, and sarcoma cell lines [49–52]. Dasatinib was shown to induce apoptosis and cell cycle arrest in mesothelioma and *EGFR*-mutant lung cancer cell lines, corresponding with decreases in STAT-3 and Akt [53, 54].

Other studies reported sensitivity to dasatinib in difficult-to-treat breast cancer cell lines displaying the triple-negative or basal subtypes [55, 56], a small tumor size and fewer metastases in pancreatic xenografts [57], and lower rates of proliferation, migration, and invasion in melanoma cell lines [58].

Safety data from phase I studies with dasatinib as monotherapy in advanced solid tumors demonstrated a maximum-tolerated dose (MTD) of 120 mg twice daily for five treatment days followed by two nontreatment days or 70 mg twice daily continuously. Major toxicities included nausea, fatigue, and rash, but the overall clinical experience indicated that dasatinib is generally well tolerated. Variable bone marrow suppression was noted, although much less so than in hematologic malignancies [59]. In combination phase I studies, dasatinib was investigated with erlotinib (an EGFR inhibitor) for NSCLC [60] and 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) plus cetuximab [61] for metastatic colon cancer, and toxicities similar to those above were shown, with headache and other gastrointestinal symptoms as additional commonly reported adverse events (AEs) (Table 1).

Though the largest body of clinical data with dasatinib is also in hematologic malignancies, promising preclinical data on solid tumor malignancies prompted many phase I and phase II solid tumor clinical studies. Ongoing trials with dasatinib as both monotherapy and in combination with other therapies are summarized in Table 2 and Table 3. With preclinical data supporting the role of Src inhibition in osteoclast suppression, it is no surprise that cancers with a predilection for bony metastases, such as prostate and breast cancer, are rational and appealing targets. A phase II study of dasatinib in chemotherapy-naïve men with castration-resistant prostate cancer demonstrated 43% and 19% stable disease (SD) rates at 12 weeks and 24 weeks of treatment, respectively. Additionally, a reduction in N-telopeptide in the urine, an accepted marker of bone resorption predictive of adverse skeletal events, was also noted in 80% of the study patients with known bony metastases [62]. A phase I/II trial of dasatinib combined with docetaxel showed no significant drug–drug interactions and manageable toxicities, and 21 of 21 patients demonstrated a partial response (PR) or SD at >6–21 weeks of follow-up [63]. These data led to the initiation of a phase III double-blinded, randomized interventional trial in this patient population comparing dasatinib plus docetaxel with placebo plus docetaxel, with a primary endpoint measure of overall survival. That study is currently recruiting. In a phase II study of dasatinib in hormone receptor (HR)⁺ advanced breast cancer, 19% of patients had controlled disease at ≥16 weeks, although significant AEs led to a dose reduction from 100 mg twice daily to 70 mg twice daily [64].

Table 1. Properties of Src/Abl inhibitors in clinical use

Compound	Structure	Kinase targets	MTD	Clinical trials	Toxicities/AEs
Bosutinib (SKI-606)	4-Anilino-3-quinolinecarbonitrile	Src, Bcr-Abl	400 mg orally daily	Phase I and II in solid tumors; phase III in hematologic malignancies	Nausea, diarrhea, anorexia, vomiting, pancytopenias
Saracatinib (AZD0530)	4-Anilinoquinazoline	Src, Bcr-Abl	175–250 mg orally daily	Phase I and II as monotherapy and in combination; multiple solid malignancies; no patient biomarker-selected trials to date	Leukopenia, neutropenia, arthralgias, rash, renal failure
Dasatinib ^a (BMS354825)	2-(Aminopyrimidinyl)thiazole-5-carboxamide	Src, Bcr-Abl, c-Kit, PDGFR, c-FMS, EphA2, SFKs	70 mg orally twice daily or 120 mg orally twice daily 5D2	Phase I and II as monotherapy and in combination; biomarker-selected trials; phase III trials in hematologic malignancies	Neutropenia, thrombocytopenia, pleural effusion, rash, gastrointestinal toxicity
Ponatinib (AP24534)	Imidazopyridazines	Src, Bcr-Abl	Undetermined	Phase I in CML/other hematological malignancies	Thrombocytopenia, headache, nausea, arthralgia, fatigue, anemia, rash, myalgia, pancreatitis
KX2-391	Small-molecule inhibitor	Selective Src, substrate-binding pocket inhibitor	40 mg orally twice daily	Phase I in advanced solid malignancies; phase II in castration resistant prostate cancer	Elevated AST/ALT, leukopenia, fatigue
XL-228	Proprietary	SFKs, IGF-1R, Aurora A, and Bcr-Abl	Undetermined	Phase I in advanced solid tumor malignancies/lymphoma and CML	Undetermined
TG100435/ TG100855	3-Aminobenzo[1,2,4] triazine	Src, Bcr-Abl	NA	Preclinical only	NA
DCC2036	Proprietary	Abl, some SFKs including Lyn and Hck	Undetermined	Phase I in leukemia, no solid tumor clinical trials	Undetermined

^aDasatinib has been U.S. Food and Drug Administration approved for the indication of second-line therapy in CML or Philadelphia chromosome positive acute lymphoblastic leukemia patients.

Abbreviations: 5D2, 5 days consecutive treatment followed by 2 nontreatment days; AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CML, chronic myelogenous leukemia; EphA2, ephrin type-A receptor 2; IGF-1R, insulin-like growth factor-1 receptor; MTD, maximum-tolerated dose; NA, not available; PDGFR, platelet-derived growth factor receptor; SFK, Src family kinase.

There have also been combination therapy trials that have shown promising results in both melanoma and colorectal cancer patients. A phase II trial of dasatinib in combination with dacarbazine in 49 patients with metastatic melanoma yielded an overall 59.2% clinical benefit rate, defined as complete response (CR) + PR + SD by the Response Evaluation Criteria in Solid Tumors, with four PRs and 25 patients with prolonged SD [65]. Src and phosphorylated (p)-Src levels in treated patient tumor blocks have not yet been reported, nor have biomarker analyses with *B-RAF* and *c-Kit* mutational status. Additional promising clinical data were observed in colorectal cancer patients who were refractory to initial FOLFOX therapy. In a phase I study of 30 patients treated with dasatinib in combination with cetuximab and FOLFOX, 24% of patients achieved a PR, including a 17% PR rate in patients previously reported to be refractory to dual therapy with FOLFOX and cetuximab [61]. These data prompted recruitment for a phase II, two-stage study that is currently under way (Tables 2 and 3).

Dasatinib as monotherapy has been less successful in early clinical trials, showing no significant clinical benefit in patients with high-grade glioma, mesothelioma, and sarcoma, despite encouraging preclinical data in these cancers. There was some benefit observed in a phase II trial studying dasatinib as first-line monotherapy for NSCLC patients, yielding a 43% disease control rate; however, this efficacy rate was lower than that of standard first-line chemotherapy. Biomarker analysis with *EGFR* and *K-RAS* mutation status was studied in these patients but did not predict response [66]. In addition to these early clinical data, phase II trials studying dasatinib as monotherapy are currently in progress for patients with advanced NSCLC, triple-negative breast cancer, head and neck squamous cell carcinoma (HNSCC), prostate cancer, and pancreatic cancer. Many phase I and phase II trials studying dasatinib in combination with other agents are also in progress for other cancers, including breast cancer, colorectal cancer, and glioblastoma (Tables 2 and 3).

Table 2. Combination clinical trials with dasatinib

Trial design	Biomarkers	Results
Phase I		
Plus FOLFOX and cetuximab in CRC patients [61] ($n = 30$)	NA	PR, 24% overall, 17% in patients refractory to FOLFOX + cetuximab alone; DCR, 56%
Neoadjuvant cetuximab versus dasatinib versus both in CRC patients with liver metastases; 4-arm study ($n = 65$); ClinicalTrials.gov Identifier, NCT00835679	p-EGFR, p-MAPK, p-Akt, Ki67, p-FAK, and others	Recruiting; estimated completion, May 2013
Plus capecitabine, oxaliplatin, and bevacizumab in CRC patients ($n = 56$); phase I/II staged trial; ClinicalTrials.gov Identifier, NCT00920868	Src downstream markers	Recruiting; estimated completion, May 2011
Plus bevacizumab in all solid tumor patients ($n = 48$); ClinicalTrials.gov Identifier, NCT00792545	Src-FAK, Src-PLC- γ , VEGF	Recruiting
Plus gemcitabine in all solid tumor patients ($n = 63$); ClinicalTrials.gov Identifier, NCT00429234	NA	Recruiting; estimated completion, January 2011
Plus bevacizumab and paclitaxel in all solid tumor patients ($n = 60$); ClinicalTrials.gov Identifier, NCT01015222	NA	Recruiting; estimated completion, November 2012
Plus dacarbazine in melanoma patients ($n = 49$) [65]	p-Src, Src levels, <i>B-Raf</i> or <i>c-Kit</i> mutations	PR, $n = 4$; pSD, $n = 25$; clinical benefit rate, 59.2%; biomarker analysis pending
Plus erlotinib in NSCLC patients ($n = 20$); ClinicalTrials.gov Identifier, NCT00444015	Antiangiogenic markers	Active, not recruiting; completion, January 2011
Plus chemoradiation in stage 3 NSCLC patients ($n = 36$); no prior chemotherapy or radiation; ClinicalTrials.gov Identifier, NCT00787852	NA	Recruiting; estimated completion, December 2010
4-arm neoadjuvant study of erlotinib, dasatinib, combination, or control in NSCLC and HNSCC patients ($n = 56$); ClinicalTrials.gov Identifier, NCT00779389	Multiple EGFR and downstream Src proteins	Recruiting; estimated completion, October 2013
Plus carboplatin and paclitaxel in ovarian, peritoneal, tubal cancer patients ($n = 24$); ClinicalTrials.gov Identifier, NCT00672295	Downstream Src substrates and microarray signatures	Active; estimated completion, September 2011
Plus radiation and temozolomide in glioblastoma patients ($n = 72$); phase I/II staged trial; ClinicalTrials.gov Identifier, NCT00895960	EphA2, c-Kit and PDGFR, PTEN	Recruiting; estimated completion, May 2012
Plus lomustine in glioblastoma patients ($n = 108$); ClinicalTrials.gov Identifier, NCT00948389	NA	Recruiting; estimated completion, January 2013
Plus paclitaxel in breast cancer patients ($n = 60$); staged phase I/II; ClinicalTrials.gov Identifier, NCT00820170	p-Src, VEGFR-2, T4 collagen, mRNA profiling	Recruiting; estimated completion, January 2013
Plus docetaxel in prostate cancer patients ($n = 66$); ClinicalTrials.gov Identifier, NCT00439270	NA	Recruiting; estimated completion, November 2010
Phase II		
Plus paclitaxel in HER-2/Neu $^{-}$, any ER breast cancer patients ($n = 23$) [94]	EphA2, IGFBP-2, caveolin, p-caveolin, p-Src, VEGFR-2, T4 collagen	Recruiting
Plus letrozole versus letrozole alone in HR $^{+}$ breast cancer patients ($n = 120$); ClinicalTrials.gov Identifier, NCT00696072	NA	Recruiting; estimated completion, August 2011
Plus exemestane versus exemestane alone in ER $^{+}$ breast cancer patients ($n = 156$); ClinicalTrials.gov Identifier, NCT00767520	NA	Recruiting; estimated completion, May 2011
Plus docetaxel in castration-resistant prostate cancer patients ($n = 46$); CA180086 trial; results led to phase III trial [63]	NA	Clinical benefit, 21 of 21 patients; at 6 wks: uPR, $n = 5$; PR, $n = 7$; SD, $n = 9$; lower PSA, 13 of 32 patients; >35% lower uNTX, 12 of 26 patients; fewer osteolytic lesions at 6 wks, 10 of 31 patients
With or without nilutamide in prostate cancer patients selected for AR activity by gene signature ($n = 60$); ClinicalTrials.gov Identifier, NCT00918385	Genetic screen AR activity	Recruiting; estimated completion, May 2011
With or without bevacizumab in glioma patients ($n = 19$) [97]	NA	No complete responses or PRs
Plus dacarbazine in melanoma patients ($n = 47$); phase I/II; no previous treatment in phase II; ClinicalTrials.gov Identifier, NCT00597038	NA	Active, not recruiting; completion, February 2011
Plus erlotinib in NSCLC, phase I/II ($n = 59$); ClinicalTrials.gov Identifier, NCT00826449	IL-8, bFGF, PDGF, VEGF	Recruiting; estimated completion, February 2015
Plus cetuximab and radiation with or without cisplatin in HNSCC patients ($n = 98$); phase I/II staged study; no previous treatment; ClinicalTrials.gov Identifier, NCT00882583	NA	Recruiting; estimated completion, January 2014
Plus ifosfamide, etoposide, and carboplatin in all solid tumor patients ($n = 143$); ClinicalTrials.gov Identifier, NCT00788125	Multiple downstream Src proteins, gene microarray	Completed, results not yet available

(continued)

Table 2. (Continued)

Trial design	Biomarkers	Results
Phase III Plus docetaxel and prednisone versus docetaxel and prednisone alone in castration-resistant prostate cancer patients ($n = 1,380$); no prior cytotoxic therapy; ClinicalTrials.gov Identifier, NCT00744497	NA	Recruiting; estimated completion, December 2012

Advanced/metastatic disease; may be previously treated unless otherwise noted.
 Abbreviations: AR, androgen receptor; bFGF, basic fibroblast growth factor; CRC, colorectal cancer; DCR, disease control rate; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FAK, focal adhesion kinase; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; HER, human epidermal growth factor receptor; HNSCC, head and neck squamous cell cancer; HR, hormone receptor; IGFBP-2, insulin growth factor binding protein 2; IL-8, interleukin 8; MAPK, mitogen-activated protein kinase; NA, not available; NSCLC, non-small cell lung cancer; p, phosphorylated; PDGF, platelet-derived growth factor; PLC- γ , phospholipase C- γ ; PR, partial response; PSA, prostate-specific antigen; pSD, prolonged stable disease; PTEN, phosphatase and tensin homologue deleted on chromosome ten; SD, stable disease; uNTX, urine N-telopeptide; uPR, unconfirmed partial response; VEGF, vascular endothelial growth factor.
 From <http://www.ClinicalTrials.gov>, accessed September 2010.

Saracatinib

Saracatinib (AZD0530; AstraZeneca, Wilmington, DE) is another orally active, highly selective, small-molecule, dual Src-Abl inhibitor that has shown promising results in preclinical and clinical studies mainly focused on solid tumors and osteolytic lesions. Antitumor effects have been observed in various solid tumor cell lines, including breast, prostate, and lung cancers. Inhibition of migration and cell invasion with saracatinib was demonstrated as well. In preclinical breast cancer studies, saracatinib in combination with antiestrogen therapy, such as tamoxifen, resulted in lower levels of Src, FAK, Akt, paxillin, CAS, cyclin D1, and c-Myc and helped prevent acquired antihormone resistance [67]. In tamoxifen-resistant breast cancer cell lines, the combination of saracatinib and gefitinib, an EGFR inhibitor added because of the higher levels of EGFR in tamoxifen-resistant cells, showed greater cell adhesion and less invasiveness [67]. Studies of prostate cancer cell lines demonstrated similarly lower levels of many of the above proteins [68]. Another study showed lower levels of interleukin 8, urokinase plasminogen activator, and MMP-9, which might retard osteolytic bone metastases. In lung cancer cell models, saracatinib inhibited downstream signaling through FAK and Akt, and demonstrated radiosensitization [69]. Results similar to those from the above studies were reported in colon cancer, head and neck cancer, and lymphoma cell lines [70–72]. Data showing the efficacy of saracatinib in reducing metastatic disease were seen in a murine metastatic model of bladder cancer in which there was a significantly lower number of tumor colonies that could be grown from mesenteric lymph node extracts in treated than in untreated mice [73].

Several phase I clinical trials of saracatinib have been conducted and an MTD of 175 mg daily has been established for advanced solid tumor malignancies. Dose-limiting toxicities

included cytopenias, asthenia, and respiratory failure [74]. Other reported mild AEs included nausea, anorexia, myalgias, cough, neutropenia, and thrombocytopenia (Table 1).

Saracatinib has had limited efficacy in phase II clinical trials in many human solid tumor malignancies. Single-agent studies in pancreatic cancer, HNSCC, HR[−] breast carcinoma, melanoma, prostate cancer, and gastric cancer patients have all been negative [75–81]. Phase II monotherapy studies currently in progress or recruiting patients include those for small cell lung cancer, metastatic colorectal cancer, sarcoma, and metastatic prostate or breast cancer with evidence of bony disease involvement. A large phase II study examining additional benefit for ovarian cancer patients treated with carboplatin and paclitaxel recently completed and was also negative [82]. Despite the lack of success in clinical trials to date, it is important to note that all clinical trials studying the efficacy of Src inhibitors have been conducted in unselected patients. Many trials with Src inhibitors, including saracatinib, are investigating possible biomarkers of response that may provide a selected population of patients who are more likely to benefit from treatment (Table 4).

Bosutinib

Bosutinib (SKI-606; Wyeth/Pfizer, New York, NY) is a 4-anilino-3-quinolinecarbonitrile and a potent, orally administered, small-molecule inhibitor of both Src and Abl tyrosine kinases. It displays 200-fold more potent inhibition of Bcr-Abl than imatinib. In breast cancer cell lines, bosutinib was shown to reduce cell proliferation, invasion, and migration by inhibiting mitogen-activated protein kinase and Akt phosphorylation [83]. In colorectal cancer, bosutinib also promoted cytosolic localization of β -catenin, leading to greater binding affinity to E-cadherin, resulting in greater adhesion of colorectal cancer cells and less mo-

Table 3. Monotherapy clinical trials with dasatinib

Study design	Biomarkers	Results
Phase I		
All solid tumor patients [98] ($n = 28$); Mesothelioma, resectable ($n = 24$); ClinicalTrials.gov Identifier, NCT00652574	NA p-Src expression	SD, $n = 6$; PR, $n = 1$ Recruiting; estimated completion, March 2011
Phase II		
Triple-negative breast cancer patients ($n = 44$); ClinicalTrials.gov Identifier, NCT00371254	NA	PR, 5%; SD > 16 wks, 5%
Advanced HR ⁺ breast cancer patients ($n = 70$) [64]	NA	PR, $n = 3$; SD > 16 wks, $n = 6$
Breast cancer patients selected based on tumor molecular features ($n = 532$); ClinicalTrials.gov Identifier, NCT00780676[99]	3 tumor groups based on pharmacogenomics	Recruiting; estimated completion, October 2024
NSCLC patients ($n = 35$); ClinicalTrials.gov Identifier, NCT00459342	NA	PR, 6%; SD, 38%
NSCLC patients ($n = 40$), ClinicalTrials.gov Identifier, NCT00858403	p-ERK, p-Akt, p-Src	Active, not recruiting; completion, April 2012
Untreated NSCLC patients ($n = 34$) [66]	<i>EGFR</i> , <i>K-RAS</i> mutations, p-SFK expression	DCR, 43%; tested markers not predictive of response
NSCLC patients ($n = 100$); ClinicalTrials.gov Identifier, NCT00787267	<i>K-RAS</i> mutation, c-terminal crosslinked collagen	Recruiting; estimated completion, January 2012
HNSCC patients ($n = 15$); ClinicalTrials.gov Identifier, NCT00507767	NA	SD > 12 wks, $n = 1$
HNSCC patients ($n = 15$); ClinicalTrials.gov Identifier, NCT00507767	Src downstream proteins, cortactin mRNA	Completed, awaiting results
Mesothelioma patients ($n = 46$) [100]	VEGF, PDGF-B, CSF-1, EphA1 mRNA, PDGFR-b,	No responses, negative study, poor tolerability
Mesothelioma patients ($n = 42$); ClinicalTrials.gov Identifier, NCT00509041	EphA2, PDGFR, VEGF, CSF-1, p-Src, p-EphA1	Active, not recruiting
Sarcoma patients, 7 histologic subtypes ($n = 189$) [101]	NA	Discontinued early because of a lack of activity
Salivary gland cancer patients ($n = 65$); ClinicalTrials.gov Identifier, NCT00859937	PDGF-A, <i>Kit</i> mutations, downstream Src proteins	Recruiting; estimated completion, August 2011
Pancreatic cancer patients ($n = 49$); ClinicalTrials.gov Identifier, NCT00474812	Undisclosed	Recruiting; estimated completion, January 2013
Pancreatic cancer patients ($n = 41$); ClinicalTrials.gov Identifier, NCT00544908	Src/STAT-3 pathway proteins	Active, not recruiting; completion, December 2010
GIST patients ($n = 52$); no prior treatment; ClinicalTrials.gov Identifier, NCT00568750	<i>Kit</i> , <i>PDGFR</i> mutation status	Recruiting; estimated completion, December 2014
Prostate cancer patients on androgen deprivation therapy ($n = 100$); no prior therapy other than antiandrogens; ClinicalTrials.gov Identifier, NCT00385580	NA	Active, not recruiting; completion, August 2010
Transitional bladder cancer patients, neoadjuvant ($n = 25$); ClinicalTrials.gov Identifier, NCT00706641	Selected biomarkers	Recruiting; estimated completion, December 2010
HCC patients ($n = 41$); ClinicalTrials.gov Identifier, NCT00459108	NA	Recruiting
CRC patients ($n = 54$); ClinicalTrials.gov Identifier, NCT00504153	Multiple SFK mutations, VEGF, p-Src, Src	Active, not recruiting
SCLC patients ($n = 56$); ClinicalTrials.gov Identifier, NCT00470054	NA	Active, not recruiting
Phase III		
None ongoing in solid tumor malignancies		

Advanced/metastatic disease; may be previously treated unless otherwise noted.

Abbreviations: CRC, colorectal cancer; CSF-1, colony stimulating factor 1; DCR, disease control rate; EphA, ephrin type-A receptor; ERK, extracellular signal-related kinase; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; HR, hormone receptor; NA, not available; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; PR, partial response; pSD, prolonged stable disease; SCLC, small cell lung cancer; SD: stable disease, SFK, Src family kinase; STAT-3, signal transducer and activator of transcription 3; uNTX, urine N-telopeptide; VEGF, vascular endothelial growth factor.

From <http://www.ClinicalTrials.gov>, accessed September 2010.

Table 4. Clinical trials with saracatinib

Study design	Biomarkers	Results
Monotherapy studies		
Phase II		
Gastric or gastroesophageal junction cancer patients ($n = 35$); ClinicalTrials.gov Identifier, NCT00607594	p-Src, CSK, p-FAK, paxillin, caveolin, STAT-3, plasma VEGF	Active, not recruiting
Locally advanced or metastatic HR ⁻ breast cancer patients ($n = 41$); ClinicalTrials.gov Identifier, NCT00559507	NA	Active, not recruiting; estimated completion, July 2010
HR ⁻ breast cancer patients ($n = 9$) [81]	IL-6, CTCs	Closed early given risk/benefit ratio; >10% treatment-related toxicity, including 1 patient with ARDS
HNSCC patients ($n = 28$); ClinicalTrials.gov Identifier, NCT00513435	NA	Active, not recruiting; estimated completion, September 2010
NSCLC patients ($n = 32$); ClinicalTrials.gov Identifier, NCT00638937	p-Src, CSK, p-FAK, paxillin, caveolin, STAT-3, plasma VEGF	Recruiting
Melanoma patients ($n = 23$) [79]	NA	No responses, no advancement to stage II arm
Melanoma patients ($n = 40$); ClinicalTrials.gov Identifier, NCT00669019	IFN- γ , p-Zap70, p-MAPK	Completed, awaiting results
Gastric cancer patients ($n = 20$) [77]	NA	pSD, $n = 1$; SD, $n = 3$; negative study, no advancement to phase II arm
Pancreatic cancer patients ($n = 18$) [80]	Gene pair analysis to identify biomarkers	No responses, no advancement to phase II arm, but biomarker selective multicenter trial in development
Saracatinib versus placebo in surgically resected patients with recurrent osteosarcoma to the lung ($n = 88$); ClinicalTrials.gov Identifier, NCT00752206	NA	Recruiting; estimated completion, September 2011
CRC patients ($n = 35$); ClinicalTrials.gov Identifier, NCT00397878	NA	Active; estimated completion, February 2011
Sarcoma patients ($n = 37$); ClinicalTrials.gov Identifier, NCT00659360	NA	Active, not recruiting
Hormone-refractory prostate cancer patients ($n = 40$); ClinicalTrials.gov Identifier, NCT00513071	NA	Completed, awaiting results
Phase III		
Currently no phase III trials		
Combination studies		
Phase I		
Plus anastrozole in HR ⁺ breast cancer patients [100]	NA	Well tolerated, phase II under way
Plus paclitaxel and/or carboplatin in solid tumor patients ($n = 234$); ClinicalTrials.gov Identifier, NCT00496028	NA	Active, not recruiting; estimated completion, October 2010
Phase II		
Plus carboplatin and paclitaxel versus carboplatin and paclitaxel alone in ovarian cancer patients ($n = 241$); ClinicalTrials.gov Identifier, NCT00610714	NA	Active; estimated completion, November 2010
Plus gemcitabine in metastatic pancreatic cancer patients ($n = 60$); phase I/II study; ClinicalTrials.gov Identifier, NCT00265876	NA	Active, not recruiting
Plus zoledronic acid in prostate or breast cancer patients with bony disease ($n = 132$); ClinicalTrials.gov Identifier, NCT00558272	NA	Active; estimated completion, July 2010
Phase III		
Currently no phase III trials		
Advanced/metastatic disease; may be previously treated unless otherwise noted. Abbreviations: ARDS, acute respiratory distress syndrome; CSK, c-terminal Src kinase; CTC, circulating tumor cells; FAK, focal adhesion kinase; HR, hormone receptor; IFN, interferon; IL-6, interleukin 6; MAPK, mitogen-activated protein kinase; NA, not available; p, phosphorylated; pSD, prolonged stable disease; SD, stable disease; STAT-3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor. From http://www.ClinicalTrials.gov , accessed September 2010.		

Table 5. Clinical trials with bosutinib in solid tumor malignancies

Monotherapy and Combination Study design	Biomarkers	Results
Phase I		
Plus gemcitabine in resected pancreatic cancer patients with no prior systemic treatment ($n = 33$); ClinicalTrials.gov Identifier, NCT01025570	NA	Recruiting; estimated completion, June 2013
Plus capecitabine in all solid tumor patients ($n = 152$); ClinicalTrials.gov Identifier, NCT00959946	NA	Recruiting; estimated completion, December 2011
Phase II		
Monotherapy in breast cancer patients ($n = 75$); ClinicalTrials.gov Identifier, NCT00319254	NA	Completed
Plus exemestane versus exemestane monotherapy in HR ⁺ breast cancer patients ($n = 234$); ClinicalTrials.gov Identifier, NCT00793546	NA	Active, not recruiting
Plus letrozole versus letrozole monotherapy in breast cancer patients ($n = 250$); ClinicalTrials.gov Identifier, NCT00880009	NA	Recruiting; estimated completion, December 2013
Phase III		
No phase III trials at present		
Advanced/metastatic disease; may be previously treated unless otherwise noted. Abbreviations: HR, hormone receptor; NA, not available. From http://www.ClinicalTrials.gov , accessed September 2010.		

tility [84]. Similarly, bosutinib demonstrated slower growth in HT29 xenograft models [85].

A phase I study of 51 patients with advanced solid tumors showed that bosutinib was generally well tolerated, with predominantly gastrointestinal AEs in 19%–69% of patients in three reports. The MTD was determined to be 400 mg daily. Study of a restricted cohort of pancreatic cancer, NSCLC, and colorectal cancer patients is ongoing [86]. Phase I/II studies in CML and Ph⁺ ALL patients who failed imatinib showed similar AEs as well as some mild dermatologic symptoms and variable hematologic toxicity [87, 88] (Table 1).

Although the body of clinical data supporting bosutinib has also been primarily applied to hematological malignancies, there are phase II trials in progress to study its efficacy in solid malignancies as well. Two phase II trials are examining HR⁺ breast cancer patients treated with an aromatase inhibitor with or without the addition of bosutinib to determine if there is added clinical benefit. Another phase II study is exploring the use of bosutinib in pancreatic adenocarcinoma in combination with gemcitabine as adjuvant therapy in patients with resected disease. Results of these studies are not yet available (Table 5).

Other Inhibitors

KX2–391 (Keryx Biopharmaceuticals; Buffalo, NY) is a novel Src inhibitor that targets the peptide substrate-binding site instead of the ATP-binding site mechanism that is common to other Src inhibitors. KX2–391 has a much wider spectrum of solid tumor activity in vitro and is more

potent in mouse xenografts than other multikinase Src/Abl inhibitors. A phase I study in advanced solid tumor malignancies demonstrated dose-limiting toxicities of elevated transaminases, neutropenia, and fatigue, all of which were reversible within 7 days. In that study, the MTD was found to be 40 mg twice daily. Phase I data in all solid tumors demonstrated biologic activity in pancreatic and prostate cancer patients, leading to a phase II trial in castration-resistant prostate cancer patients [89] (Table 6). Of note, KX2–391 also inhibits microtubule polymerization, so the mechanism of action is somewhat unclear.

XL228 (Exelixis Pharmaceuticals, San Francisco, CA) is currently being studied in a phase I trial for the treatment of patients with CML or Ph⁺ ALL as well in a phase I trial for patients with all other types of unresectable, advanced solid tumor malignancies and lymphoma (Table 6).

Other inhibitors in development, which are in preclinical stages only, include DCC-2036 (Deciphera Pharmaceuticals, Lawrence, KS), an Abl inhibitor that also inhibits two of the SFKs; ponatinib (AP24534; ARIAD Pharmaceuticals, Inc., Cambridge, MA), the phase I results of which were recently presented [90]; and TG100435 (TargeGen, San Diego, CA), a 3-aminobenzotriazine compound (Table 6).

BIOMARKERS IN SRC-TARGETED THERAPIES

Biomarkers predictive of response to a targeted therapy are an attractive area of investigation because these agents have limited success in unselected populations. Although many downstream effectors of the Src pathway appear to be

Table 6. Clinical trials with other Src inhibitors

Drug	Study design		Biomarkers	Status
XL-999	Phase I	NSCLC, CRC, RCC, and ovarian cancer patients	NA	Discontinued because of cardiotoxicity of drug
KX2-391	Phase I; phase II	Patients with metastatic solid tumors refractory to conventional therapies ($n = 50$); ClinicalTrials.gov Identifier, NCT00646139; bone-metastatic prostate cancer, castration-resistant prostate cancer; no prior treatment; ClinicalTrials.gov Identifier, NCT01074138	Plasma VEGF, p-Src NA	Active, not recruiting; recruiting
XL-228	Phase I	Advanced solid tumor patients ($n = 100$); ClinicalTrials.gov Identifier, NCT00526838	NA	Active, not recruiting
TG100435/TG100855	Preclinical	Preclinical studies only	NA	NA

Diseases advanced/metastatic and may be previously treated unless otherwise noted
Abbreviations: CRC, colorectal cancer; NA, not applicable; NSCLC, non-small cell lung cancer; RCC, renal cell cancer; VEGF, vascular endothelial growth factor.
From <http://www.ClinicalTrials.gov>, accessed September 2010.

downregulated with treatment in cell line models, whether these reductions translate into treatment benefit is unclear. Preclinical studies on papillary and anaplastic thyroid cancer cell lines showed no correlation between p-Src levels and response to saracatinib, but high levels of p-FAK were strongly correlated with saracatinib sensitivity. These data suggest that p-FAK may be a better predictive biomarker in thyroid cancer than p-Src [91]. In the clinical arena, biomarkers established in preclinical work are currently being measured in pre- and post-treatment samples to determine whether high expression in pretreatment patients predicts response and whether that response is correlated with a corresponding decrease in biomarker levels. These results hopefully will identify subpopulations who are more likely to benefit from treatment. Most of the clinical trials incorporating a biomarker investigation are studies with dasatinib. Candidate biomarkers in these trials include STAT-3, cortactin, c-Kit, b-Raf, VEGF, CSF-1, EphA1 mRNA, EphA2, p-Src, and other phosphorylated downstream SFK effectors in various cancers. Biomarker investigations in clinical trials with dasatinib and saracatinib are summarized in Tables 2–4.

Other studies are screening for genetic markers as predictors of response. Those studies also primarily examined dasatinib. To date, clinical trials of selected patients based on such genetic biomarkers include a breast cancer trial of dasatinib in selected patients based on gene-expression profiling of three distinct biomarkers that were identified in sensitive versus resistant breast cancer biopsy specimens [92]. Similarly, a phase II study in prostate cancer patients is under way that selects patients based on genomic evalu-

ation of androgen receptor (AR) activity, whereby patients with high AR profiles are assigned to hormonal therapy with nilutamide and those with low AR profiles are assigned to dasatinib. That trial is based on preclinical data on prostate cancer cell lines and tumor biopsy specimens suggesting that lower AR activity was correlated with sensitivity to dasatinib [93]. Additionally, preclinical data with pancreatic xenografts using a *k*-top scoring pairs classifier method to identify a gene pair predictive of saracatinib sensitivity led to the development of a phase II clinical trial selecting patients with this particular gene pair signature [45]. Other clinical trials are also using microarrays to identify candidate genes that might predict response.

CONCLUSION

As the oldest known proto-oncogene, Src has been extensively studied and is involved in numerous intracellular processes that are important in cancer growth, progression, motility, invasion, and metastasis. Although Src/Abl inhibitors have found their way to the clinic via Abl inhibition in CML, thus far their activity in solid tumors has been limited. Several Src inhibitors are in phase II/III clinical trials in solid tumor patients, with particular promise noted in castration-resistant prostate cancer. The future likely lies in combination strategies and biomarker-driven studies.

AUTHOR CONTRIBUTIONS

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REFERENCES

- 1 Rous P. A sarcoma of the fowl transmissible by an agent separable from the tumors cells. *J Exp Med* 1911;13:397–411.
- 2 Rubin H. Quantitative relations between causative virus and cell in the Rous no. 1 chicken sarcoma. *Virology* 1955;1:445–473.
- 3 Martin GS. Rous sarcoma virus: A function required for the maintenance of the transformed state. *Nature* 1970;227:1021–1023.
- 4 Stehelin D, Varmus HE, Bishop JM et al. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 1976;260:170–173.
- 5 Stehelin D, Guntaka RV, Varmus HE et al. Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. *J Mol Biol* 1976;101:349–365.
- 6 Martin GS. The road to Src. *Oncogene* 2004;23:7910–7917.
- 7 Yeatman TJ. A renaissance for Src. *Nat Rev Cancer* 2004;4:470–480.
- 8 Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol* 1997;13:513–609.
- 9 Bolen JB, Brugge JS. Leukocyte protein tyrosine kinases: Potential targets for drug discovery. *Annu Rev Immunol* 1997;15:371–404.
- 10 Roskoski R Jr. Src protein-tyrosine kinase structure and regulation. *Biochem Biophys Res Commun* 2004;324:1155–1164.
- 11 Manning G, Whyte DB, Martinez R et al. The protein kinase complement of the human genome. *Science* 2002;298:1912–1934.
- 12 Haskell MD, Slack JK, Parsons JT et al. c-Src tyrosine phosphorylation of epidermal growth factor receptor, P190 RhoGAP, and focal adhesion kinase regulates diverse cellular processes. *Chem Rev* 2001;101:2425–2440.
- 13 Brown MT, Cooper JA. Regulation, substrates, and functions of Src. *Biochim Biophys Acta* 1996;1287:121–149.
- 14 Fujita Y, Krause G, Scheffner M et al. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. *Nat Cell Biol* 2002;4:222–231.
- 15 Zamir E, Geiger B. Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci* 2001;114:3583–3590.
- 16 Noritake H, Miyamori H, Goto C et al. Overexpression of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) in metastatic MDCK cells transformed by v-Src. *Clin Exp Metastasis* 1999;17:105–110.
- 17 Hsia DA, Mitra SK, Hauck CR et al. Differential regulation of cell motility and invasion by FAK. *J Cell Biol* 2003;160:753–767.
- 18 Niu G, Wright KL, Huang M et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene* 2002;21:2000–2008.
- 19 Parsons JT, Parsons SJ. Src family protein tyrosine kinases: Cooperating with growth factor and adhesion signaling pathways. *Curr Opin Cell Biol* 1997;9:187–192.
- 20 Courtneidge S, Fumagalli S, Koegl M et al. The Src family of protein tyrosine kinases: Regulation and functions. *Dev Suppl* 1993;57–64.
- 21 LaVallee TM, Prudovsky IA, McMahon GA et al. Activation of the MAP kinase pathway by FGF-1 correlates with cell proliferation induction while activation of the Src pathway correlates with migration. *J Cell Biol* 1998;141:1647–1658.
- 22 Levitzki A. Src as a target for anti-cancer drugs. *Anticancer Drug Des* 1996;11:175–182.
- 23 Luttrell DK, Lee A, Lansing TJ et al. Involvement of pp60c-Src with two major signaling pathways in human breast cancer. *Proc Natl Acad Sci U S A* 1994;91:83–87.
- 24 Rahimi N, Hung W, Tremblay E et al. c-Src kinase activity is required for hepatocyte growth factor-induced motility and anchorage-independent growth of mammary carcinoma cells. *J Biol Chem* 1998;273:33714–33721.
- 25 Tice DA, Biscardi JS, Nickles AL et al. Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc Natl Acad Sci U S A* 1999;96:1415–1420.
- 26 Peterson JE, Kulik G, Jelinek T et al. Src phosphorylates the insulin-like growth factor type I receptor on the autophosphorylation sites. Requirement for transformation by Src. *J Biol Chem* 1996;271:31562–31571.
- 27 Alvarez RH, Kantarjian HM, Cortes JE. The role of Src in solid and hematologic malignancies: Development of new-generation Src inhibitors. *Cancer* 2006;107:1918–1929.
- 28 Mao W, Irby R, Coppola D et al. Activation of c-Src by receptor tyrosine kinases in human colon cancer cells with high metastatic potential. *Oncogene* 1997;15:3083–3090.
- 29 Maa MC, Leu TH, McCarley DJ et al. Potentiation of epidermal growth factor receptor-mediated oncogenesis by c-Src: Implications for the etiology of multiple human cancers. *Proc Natl Acad Sci U S A* 1995;92:6981–1985.
- 30 Xing C, Imagawa W. Altered MAP kinase (ERK1,2) regulation in primary cultures of mammary tumor cells: Elevated basal activity and sustained response to EGF. *Carcinogenesis* 1999;20:1201–1208.
- 31 Salh B, Marotta A, Matthewson C et al. Investigation of the Meh-MAP kinase-Rsk pathway in human breast cancer. *Anticancer Res* 1999;19:731–740.
- 32 Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev* 2003;22:337–358.
- 33 Irby RB, Yeatman TJ. Role of Src expression and activation in human cancer. *Oncogene* 2000;19:5636–5642.
- 34 Bolen JB, Veillette A, Schwartz AM et al. Analysis of pp60c-Src in human colon carcinoma and normal human colon mucosal cells. *Oncogene Res* 1987;1:149–168.
- 35 Talamonti MS, Roh MS, Curley SA et al. Increase in activity and level of pp60c-Src in progressive stages of human colorectal cancer. *J Clin Invest* 1993;91:53–60.
- 36 Park J, Meisler AI, Cartwright CA. c-Yes tyrosine kinase activity in human colon carcinoma. *Oncogene* 1993;8:2627–2635.
- 37 Cattan N, Rochet N, Mazeau C et al. Establishment of two new human bladder carcinoma cell lines, CAL 29 and CAL 185. Comparative study of cell scattering and epithelial to mesenchyme transition induced by growth factors. *Br J Cancer* 2001;85:1412–1417.
- 38 Kumble S, Omary MB, Cartwright CA et al. Src activation in malignant and premalignant epithelia of Barrett's esophagus. *Gastroenterology* 1997;112:348–356.
- 39 Jankowski J, Coghill G, Hopwood D et al. Oncogenes and onco-suppressor gene in adenocarcinoma of the oesophagus. *Gut* 1992;33:1033–1038.
- 40 Mazurenko NN, Kogan EA, Zborovskaya IB et al. Expression of pp60c-Src in human small cell and non-small cell lung carcinomas. *Eur J Cancer* 1992;28:372–377.
- 41 Rosen N, Bolen JB, Schwartz AM et al. Analysis of pp60c-Src protein kinase activity in human tumor cell lines and tissues. *J Biol Chem* 1986;261:13754–13759.
- 42 Jacobs C, Rubsamen H. Expression of pp60c-Src protein kinase in adult and fetal human tissue: High activities in some sarcomas and mammary carcinomas. *Cancer Res* 1983;43:1696–1702.
- 43 Tan M, Li P, Klos KS et al. ErbB2 promotes Src synthesis and stability:

- Novel mechanisms of Src activation that confer breast cancer metastasis. *Cancer Res* 2005;65:1858–1867.
- 44 Lutz MP, Esser IB, Flossmann-Kast BB et al. Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem Biophys Res Commun* 1998;243:503–508.
 - 45 Rajeshkumar NV, Tan AC, De Oliveira E et al. Antitumor effects and biomarkers of activity of AZD0530, a Src inhibitor, in pancreatic cancer. *Clin Cancer Res* 2009;15:4138–4146.
 - 46 Messersmith WA, Rajeshkumar NV, Tan AC et al. Efficacy and pharmacodynamic effects of bosutinib (SKI-606), a Src/Abl inhibitor, in freshly generated human pancreas cancer xenografts. *Mol Cancer Ther* 2009;8:1484–1493.
 - 47 Flossmann-Kast BB, Jehle PM, Hoeflich A et al. Src stimulates insulin-like growth factor I (IGF-I)-dependent cell proliferation by increasing IGF-I receptor number in human pancreatic carcinoma cells. *Cancer Res* 1998;58:3551–3554.
 - 48 Kantarjian H, Shah NP, Hochhaus A et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2010;362:2260–2270.
 - 49 Shor AC, Keschman EA, Lee FY et al. Dasatinib inhibits migration and invasion in diverse human sarcoma cell lines and induces apoptosis in bone sarcoma cells dependent on SRC kinase for survival. *Cancer Res* 2007;67:2800–2808.
 - 50 Nam S, Kim D, Cheng JQ et al. Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res* 2005;65:9185–9189.
 - 51 Serrels A, Macpherson IR, Evans TR et al. Identification of potential biomarkers for measuring inhibition of Src kinase activity in colon cancer cells following treatment with dasatinib. *Mol Cancer Ther* 2006;5:3014–3022.
 - 52 Johnson FM, Saigal B, Talpaz M et al. Dasatinib (BMS-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non-small cell lung cancer cells. *Clin Cancer Res* 2005;11:6924–6932.
 - 53 Tsao AS, He D, Saigal B et al. Inhibition of c-Src expression and activation in malignant pleural mesothelioma tissues leads to apoptosis, cell cycle arrest, and decreased migration and invasion. *Mol Cancer Ther* 2007;6:1962–1972.
 - 54 Song L, Morris M, Bagui T et al. Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival. *Cancer Res* 2006;66:5542–5548.
 - 55 Tryfonopoulos D, O'Donovan N, Corkery B et al. Activity of dasatinib with chemotherapy in triple-negative breast cancer cells. *J Clin Oncol* 2009;27(15 suppl):Abstract e14605.
 - 56 Huang F, Reeves K, Han X et al. Identification of candidate molecular markers predicting sensitivity in solid tumors to dasatinib: Rationale for patient selection. *Cancer Res* 2007;67:2226–2238.
 - 57 Trevino JG, Summy JM, Lesslie DP et al. Inhibition of Src expression and activity inhibits tumor progression and metastasis of human pancreatic adenocarcinoma cells in an orthotopic nude mouse model. *Am J Pathol* 2006;168:962–972.
 - 58 Eustace AJ, Crown J, Clynes M et al. Preclinical evaluation of dasatinib, a potent Src kinase inhibitor, in melanoma cell lines. *J Transl Med* 2008;6:53.
 - 59 Demetri GD, Lo Russo P, MacPherson IR et al. Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors. *Clin Cancer Res* 2009;15:6232–6240.
 - 60 Haura EB, Tanvetayan T, Chiappori A et al. Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2010;28:1387–1394.
 - 61 Lieu CH, Wolff RA, Eng C. Phase IB study of the Src inhibitor dasatinib with FOLFOX and cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2010;28(15 suppl):Abstract 3536.
 - 62 Yu EY, Wilding G, Posadas E et al. Phase II study of dasatinib in patients with metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2009;15:7421–7428.
 - 63 Araujo J, Armstrong A, Braud EL et al. Dasatinib and docetaxel combination treatment for patients with castration-resistant progressive prostate cancer: A phase I/II study (CA180086). *J Clin Oncol* 2009;27(15 suppl):Abstract 5061.
 - 64 Mayer E, Baurain J, Sparano J et al. Dasatinib in advanced HER2/neu amplified and ER/PR-positive breast cancer: Phase II study CA180088. *J Clin Oncol* 2009;27(15 suppl):Abstract 1011.
 - 65 Algazi AP, Weber JS, Andrews S et al. A phase I/II trial of DTIC and dasatinib in metastatic melanoma. *J Clin Oncol* 2010;28(15 suppl):Abstract 8532.
 - 66 Johnson FM, Bekele BN, Feng L et al. Phase II study of dasatinib in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2010;28:4609–4615.
 - 67 Hiscox S, Morgan L, Green TP et al. Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells. *Breast Cancer Res Treat* 2006;97:263–274.
 - 68 Chang YM, Bai L, Liu S et al. Src family kinase oncogenic potential and pathways in prostate cancer as revealed by AZD0530. *Oncogene* 2008;27:6365–6375.
 - 69 Purnell PR, Mack PC, Tepper CG et al. The Src inhibitor AZD0530 blocks invasion and may act as a radiosensitizer in lung cancer cells. *J Thorac Oncol* 2009;4:448–454.
 - 70 Koppikar P, Choi SH, Egloff AM et al. Combined inhibition of c-Src and epidermal growth factor receptor abrogates growth and invasion of head and neck squamous cell carcinoma. *Clin Cancer Res* 2008;14:4284–4291.
 - 71 Nowak D, Boehrer S, Hochmuth S et al. Src kinase inhibitors induce apoptosis and mediate cell cycle arrest in lymphoma cells. *Anticancer Drugs* 2007;18:981–995.
 - 72 Arcaroli JJ, Touban BM, Tan AC et al. Gene array and fluorescence in situ hybridization biomarkers of activity of saracatinib (AZD0530), a Src inhibitor, in a preclinical model of colorectal cancer. *Clin Cancer Res* 2010;16:4165–4177.
 - 73 Green TP, Fennell M, Whittaker R et al. Preclinical anticancer activity of the potent, oral Src inhibitor AZD0530. *Mol Oncol* 2009;3:248–261.
 - 74 Baselga J, Cervantes A, Martinelli E et al. Phase I safety, pharmacokinetics, and inhibition of SRC activity study of saracatinib in patients with solid tumors. *Clin Cancer Res* 2010;16:4876–4883.
 - 75 Renouf DJ, Moore MJ, Hedley D et al. A phase I/II study of the Src inhibitor saracatinib (AZD0530) in combination with gemcitabine in advanced pancreatic cancer. *Invest New Drugs* 2010 Dec 18 [Epub ahead of print].
 - 76 Fury MG, Baxi S, Shen R et al. Phase II study of saracatinib (AZD0530) for patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC). *Anticancer Res* 2011;31:249–253.
 - 77 Mackay HJ, Au HJ, McWhirter E et al. A phase II trial of the Src kinase inhibitor saracatinib (AZD0530) in patients with metastatic or locally advanced gastric or gastro esophageal junction (GEJ) adenocarcinoma: A trial of the PMH phase II consortium. *Invest New Drugs* 2011 Mar 12 [Epub ahead of print].

- 78 Lara PN Jr, Longmate J, Evans CP et al. A phase II trial of the Src-kinase inhibitor AZD0530 in patients with advanced castration-resistant prostate cancer: A California Cancer Consortium study. *Anticancer Drugs* 2009; 20:179–184.
- 79 Gajewski T, Zha Y, Clark J. Phase II study of the Src family kinase inhibitor saracatinib (AZD0530) in metastatic melanoma. *J Clin Oncol* 2010; 28(15 suppl):Abstract 8562.
- 80 Messersmith WA, Nallapareddy S, Arcaroli J et al. A phase II trial of saracatinib (AZD0530), an oral Src inhibitor, in previously treated metastatic pancreatic cancer. *J Clin Oncol* 2010;28(suppl):Abstract e14515.
- 81 Traina TA, Sparano JA, Caravelli J et al. Phase II trial of saracatinib in patients (pts) with ER/PR-negative metastatic breast cancer (MBC). *J Clin Oncol* 2010;28(15 suppl):Abstract 1086.
- 82 Poole C, Lisyanskaya A, Rodenhuis S et al. A randomized phase II clinical trial of the Src inhibitor saracatinib (AZD0530) with carboplatin + paclitaxel vs. placebo, carboplatin + paclitaxel in patients with recurrent platinum-sensitive epithelial ovarian cancer. *Ann Oncol* 2010;21(suppl 8): 9720.
- 83 Jallal H, Valentino ML, Chen G et al. A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo. *Cancer Res* 2007;67:1580–1588.
- 84 Coluccia AM, Benati D, Dekhil H et al. SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60(c-Src)-dependent tyrosine phosphorylation of beta-catenin and its nuclear signaling. *Cancer Res* 2006;66:2279–2286.
- 85 Golas JM, Lucas J, Etienne C et al. SKI-606, a Src/Abl inhibitor with in vivo activity in colon tumor xenograft models. *Cancer Res* 2005;65: 5358–5364.
- 86 Messersmith WA, Krishnamurthi S, Hewes BA et al. Bosutinib (SKI-606), a dual Src/Abl tyrosine kinase inhibitor: Preliminary results from a phase I study in patients with advanced malignant solid tumors. *J Clin Oncol* 2007;25(18 suppl):Abstract 3552.
- 87 Gambacorti-Passerini C, Kantarjian H, Baccarani M et al. Activity and tolerance of bosutinib in patients with AP and BP CML and Ph⁺ ALL. *J Clin Oncol* 2008;26(suppl):Abstract 7049.
- 88 Bruemendorf TH, Cervantes F, Kim D et al. Bosutinib is safe and active in patients with chronic phase CML with resistance or intolerance to imatinib and other tyrosine kinase inhibitors. *J Clin Oncol* 2008;26(suppl): Abstract 7001.
- 89 Adjei AA, Cohen R, Kurzrock R et al. Results of a phase I trial of KX2-391, a novel non-ATP competitive substrate-pocket directed SRC inhibitor, in patients with advanced malignancies. *J Clin Oncol* 2009;27(15 suppl):Abstract 3511.
- 90 Cortes J, Talpaz M, Bixby D et al. A phase I trial of oral ponatinib (AP24534) in patients with refractory chronic myelogenous leukemia (CML) and other hematologic malignancies: Emerging safety and clinical response findings. Presented at the American Society of Hematology 2010 Annual Meeting.
- 91 Schweppe RE, Kerege AA, French JD et al. Inhibition of Src with AZD0530 reveals the Src-Focal Adhesion kinase complex as a novel therapeutic target in papillary and anaplastic thyroid cancer. *J Clin Endocrinol Metab* 2009;94:2199–2203.
- 92 Moulder S, Yan K, Huang F et al. Development of candidate genomic markers to select breast cancer patients for dasatinib therapy. *Mol Cancer Ther* 2010;9:1120–1127.
- 93 Mendiratta P, Mostaghel E, Guinney J et al. Genomic strategy for targeting therapy in castration-resistant prostate cancer. *J Clin Oncol* 2009;27: 2022–2029.
- 94 Morris PG, Chang JC, Abbruzzi A et al. Correlative biomarkers in a phase II study of dasatinib (D) and weekly (w) paclitaxel (P) for patients (Pts) with metastatic breast carcinoma (MBC). *J Clin Oncol* 2010;28(15 suppl): Abstract TPS124.
- 95 Lu-Emerson C, Norden AD, Drappatz J et al. Retrospective study of dasatinib in recurrent high-grade glioma (HGG) patients who failed bevacizumab. *J Clin Oncol* 2010;28(suppl):Abstract e12525.
- 96 Johnson FM, Agrawal S, Burris H et al. Phase I pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors. *Cancer* 2010;116:1582–1591.
- 97 Pusztai L, Moulder SL, Litton JK et al. Gene-signature-based patient selection for dasatinib therapy in metastatic breast cancer (MBC). *J Clin Oncol* 2010;28(15 suppl):Abstract TPS130.
- 98 Tsao AS, Wistuba II, Mehran RJ. Evaluation of Src Tyr419 as a predictive biomarker in a neoadjuvant trial using dasatinib in resectable malignant pleural mesothelioma. *J Clin Oncol* 2010;28(15 suppl):Abstract 7042.
- 99 Schuetze S, Wathen K, Choy E et al. Results of a Sarcoma Alliance for Research through Collaboration (SARC) phase II trial of dasatinib in previously treated, high-grade, advanced sarcoma. *J Clin Oncol* 2010;28(15 suppl):Abstract 10009.
- 100 Pegram MD, Silva OE, Higgins C et al. Phase IB pharmacokinetic (PK) study of Src kinase inhibitor AZD0530 plus anastrozole in postmenopausal hormone receptor positive (HR⁺) metastatic breast cancer (MBC). *J Clin Oncol* 2010;28(suppl):Abstract e13074.